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WHITE PAPER

Microba's Science Review Process

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Overview

The number of scientific studies published on the gut microbiome is rapidly increasing every year. However, the quality of research varies, and it can be difficult to sort through claims supported by good quality scientific evidence compared to poor quality evidence.

To assist healthcare professionals in evaluating the current evidence on the gut microbiome, Microba developed a Science Review Process (SRP) based on evidence-based medicine (EBM) principles. The SRP is used to evaluate the quality of evidence available to support health associations and interventions for microbial and gastrointestinal markers that are assessed in Microba's microbiome testing products.

Microbial markers provide information on the relative abundance or proportion of microbial cells that have the genetic capacity to produce or consume the named marker. Gastrointestinal health markers provide measures of human markers in the stool.

The Microba SRP was applied in an extensive review for the MetaXplore range of tests over a period of eight months in 2021-2022. The review covered the association of gut microbial markers and gastrointestinal health markers with selected health categories, and interventions to address altered microbial and gastrointestinal health markers. Subsequent reviews are to be conducted on an annual basis to update statements and grades with new evidence.

Evidence-based medicine

Evidence-based medicine (EBM) is defined as "the conscientious, explicit, judicious and reasonable use of current best evidence in making decisions about the care of individual patients[1]." It is a process for systematically locating, appraising, and applying research findings to inform clinical decisions and consists of the following steps[2]:

1) Define the clinically relevant research question
2) Search the scientific literature for relevant articles
3) Critically evaluate the evidence for its validity and usefulness
4) Implement useful findings in clinical practice

Microba's SRP applies the first three steps of this process to develop statements about the available evidence for an association between a marker and a health category or to support an intervention to alter a marker.

Several EBM systems have been developed to address step 3, the critical evaluation of available evidence for its validity and usefulness. In general, these EBM systems apply the same hierarchy of evidence, where the strongest evidence is from randomised, placebo-controlled trials and the weakest evidence is from case series or pre-post measurements (**Figure 1**).

To determine the best EBM system to use for the SRP, Microba reviewed the following systems: GRADE (Grading of Recommendations, Assessment, Development, and Evaluations)[3], SIGN (Scottish Intercollegiate

Guidelines Network)[4], GATE (Graphic Appraisal Tool for Epidemiology)[5], OCEBM (Oxford Centre for Evidence-Based Medicine)[6], and the NHMRC (National Health and Medical Research Council) Levels of Evidence[8]. After reviewing the different systems, Microba chose to adopt the NHMRC Levels of Evidence because of its applicability to a wide range of microbiome-based research questions, its ease of implementation compared to other EBM systems, and its derivation from the highly recognised SIGN and OCEBM systems[7].

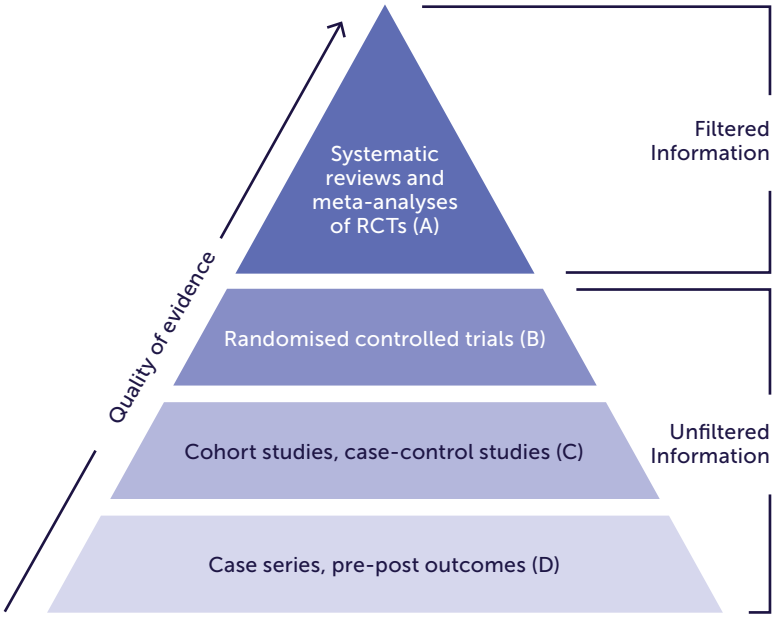

Figure 1. Level of evidence hierarchy used in Evidence-Based Medicine.

A closer look at the NHMRC Levels of Evidence

Microba uses the NHMRC Levels of Evidence to critically appraise the body of evidence for a specific research question. This method rates five key components[8]:

- Evidence base: consists of three areas – quantity of evidence, level of evidence (study design – **Figure 1**), and quality of evidence (risk of bias analysis)
- Consistency of evidence
- Potential clinical impact
- Generalisability of the available evidence
- Applicability of the available evidence

Each of these areas are assigned a grade from A to D. The individual ratings for each of the five components are then summed to provide an overall grade. The overall grade is intended to indicate the strength of the evidence that supports a statement, with "A" being the strongest level of evidence and "D" being the weakest (**Table 1**).

When determining the overall grade, the components "evidence base" and "consistency of evidence" play the largest role, as an overall evidence grade of A or B cannot be assigned unless both of these components are rated A or B[8].

Table 1. Definition of NHMRC grades of recommendations

Grade	Description
A	Body of evidence can be trusted to guide practice
B	Body of evidence can be trusted to guide practice in most situations
C	Body of evidence provides some support for recommendation, but care should be taken in its application
D	Body of evidence is weak and recommendation must be applied with caution

Microba's Science Review Process in practice

Microba used the SRP for an intensive review of the scientific literature for the MetaXplore range of tests over an eight-month period from September 2021 – April 2022. Microba's SRP followed five main steps:

1) **Define the clinical question.** For example, "What interventions are available to address low butyrate?"
2) **Conduct a high-level review** of the information available for the topic.
3) **Define specific research questions.** The information available for a topic is reviewed and specific research questions are identified where there is likely to be evidence available. For example, "Is resistant starch type 2 effective at increasing butyrate production?"
4) **Critically review the available scientific evidence.** Each study that addresses the research question is assessed for its design (level of evidence), risk of bias (**Table 2**), and results by at least one science reviewer. Once the reviewer believes all available studies for a question have been assessed, they review the body of evidence as a whole and propose an evidence grade using the NHMRC Levels of Evidence. At least 10% of reviews are independently replicated by a second reviewer to ensure consistency.
5) **Workshop findings and assign an evidence grade.** Workshops are held with a minimum of three scientific reviewers. If more than one reviewer graded the evidence for a question, both reviewers present their findings. All reviewers ask questions about the studies, considering the methods used, clinical relevance, and applicability. The proposed grade is then discussed until a consensus is reached. Based on the evidence grade, a statement summarising the research findings is developed using NICE guidelines[9]. For statements that do not have human evidence to support them (such as how microbial metabolites may influence the immune system), proposed mechanisms of action based on in vitro and/or animal studies are reviewed, however no evidence grade is assigned. In this case, these statements are classified as "practice points" and statements specify the type of study (e.g., in vitro) used for the evidence base.

Table 2. Risk of Bias (RoB) tools used for various study designs

Study Design	RoB Tool
Randomised Controlled Trials (RCTs)	Cochrane RoB 2.0[10]
Randomised Crossover Trials	Cochrane RoB Crossover variant[11]
Non-Randomised Controlled Trials (NRCTs)	ROBINS-I[12]
Systematic Reviews	ROBIS[13]
Cohort Studies	OSQE[14] or NOS[15]
Case-control	OSQE[14] or NOS[15]
Cross-sectional	OSQE[14]

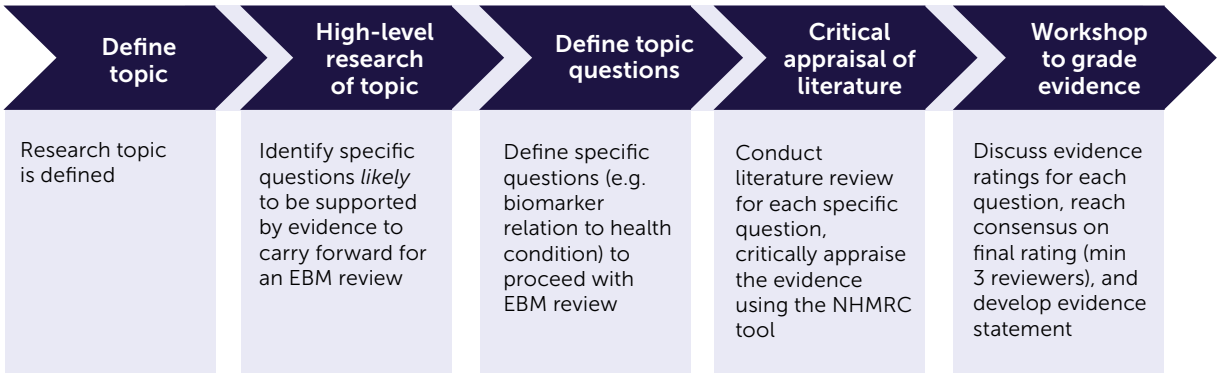

Figure 2. Microba's Science Review Process

Seven scientific reviewers were used in the SRP during the initial MetaXplore review. They were selected for their expertise in one of the following areas: gut microbiome science, molecular biology, nutrition, dietetics, food science, naturopathy, and/or evidence-based medicine. Five of the seven reviewers held a PhD and four were practicing healthcare professionals. All reviewers were trained in the use of the NHMRC levels of evidence and the use of risk of bias analysis tools. See **Appendix A** for further details on the 2021-2022 and 2024 Microba scientific reviewers.

The scientific reviewers evaluated the quality of evidence to support health associations and interventions for microbial and gastrointestinal health markers. Health associations were assessed for six general health categories: intestinal inflammation, intestinal barrier, systemic inflammation, intestinal motility, digestive secretions and detox/retox. Before starting the review, acceptable clinical measures were identified for each health category that could be used to validate associations with the microbial or gastrointestinal health marker (**Table 3**). Health associations of markers were graded on the quality of evidence to support a positive or negative correlation with the specified clinical measure.

Table 3. Clinical markers used to validate the association of a gut microbial or gastrointestinal health marker and a health category.

Health category	Clinical measure
Intestinal inflammation	Calprotectin, lactoferrin, endoscopy and histology
Intestinal barrier	Dual sugar test, histology
Systemic inflammation	CRP, hsCRP, WBC, SAA, TNF-a, IL-6, IL-8, GlycA
Intestinal motility	Transit study using radiopaque markers
Digestive secretions	Secretin pancreatic function test
Detox/retox	Urinary oxalate excretion/ risk of kidney stones, faecal beta-glucuronidase enzymatic activity.

When human evidence was not available, but compelling in vitro and/or animal data were available, statements were not assigned a grade and instead labelled as a "practice point" (PP). This occurred in the following scenarios:

1) Statements regarding the association of a microbial marker with a health category e.g. in vitro evidence for a mechanism of action, that may also include validation of the mechanism in animal models). (PP, IV)
2) Interventions based on associations in human cross-sectional studies (e.g., association of a microbial metabolite with altered consumption of specific foods or diets). (PP, H)
3) Interventions based on in vitro fermentation studies using human stool (e.g., evidence that incubation of a specific prebiotic fibre with stool samples results in the production of butyrate). (PP, IV)

The process to develop statements based on in vitro and/or animal data followed the same process as described above (**Figure 2**), except for the assignment of an evidence grade. The workshop discussion focused instead on the clinical need for the statement and if the in vitro and/or animal evidence was of high enough quality to justify a statement. If a consensus was reached to include the data, a statement was drafted and voted on by the review team. A minimum of 80% agreement was required to include the statement. If this was not reached, the statement was re-drafted until it met the 80% threshold.

Results from the initial SRP for MetaXplore™

During the initial eight-month intensive science review period, the science review team spent over 7000 hours assessing the evidence from over 1200 scientific publications to address approximately 300 research questions. Reviews were duplicated for 11% of the research questions and proposed NHMRC levels of evidence grades were the same 84% of the time among duplicate reviewers. As described above, final evidence grades and statements were discussed among the scientific reviewers until a consensus was reached. This review resulted in 130 evidence statements (**Table 4**), as follows:

- 15 graded statements and 12 practice points linking 14 microbial markers to five health categories.
- 23 graded statements and two practice points linking seven gastrointestinal health markers to four health categories.
- 60 graded statements and 18 practice points addressing interventions for altering markers. These included:
    - 19 graded clinical insights (scientifically graded practice recommendations to address GI health markers)
    - 41 graded research insights (scientifically graded research insights to address microbial markers)
    - 18 practice point research insights (non-graded research insights to address microbial markers)

Table 4. Examples of graded and practice point statements for the association of microbial and gastrointestinal health markers with a health category, and for interventions to address out of range markers. PP = practice point. IV = in vitro. H = human.

Marker	Evidence statement	Grade
Hexa-acylated lipopolysaccharides (hexa-LPS) producing microbes	Studies in human cell lines and animals suggest that hexa-LPS promotes intestinal inflammation through the activation of the immune receptor TLR4.	PP, IV
Methane producing archaea	A slower gut transit time and/or constipation may be associated with higher methane production.	C
Trimethylamine producing microbes	Higher levels of plasma trimethylamine-N-oxide are associated with systemic inflammation, especially in patients with type 2 diabetes and cardiovascular disease.	B
Calprotectin	Faecal calprotectin has a high sensitivity and lower specificity for identifying inflammation in IBD. Faecal calprotectin performs better in ulcerative colitis than in Crohn's disease.	A
Pancreatic elastase	Pancreatic elastase testing may be a valid method for detecting severe pancreatic insufficiency. Elastase-1 may be an inaccurate marker for ruling out mild-moderate pancreatic insufficiency.	C
Butyrate producing microbes	Resistant starch type 2 supplementation may increase butyrate producing microbes.	C
Indole-propionic acid (IPA) producing microbes	Observational studies have shown an association between intake of wholegrain wheat and rye and higher plasma IPA.	PP, H
Secretory IgA	Consider GOS (galacto-oligosaccharides) supplementation to increase faecal secretory IgA.	C

Future use

Microba's SRP was established to provide transparency to healthcare professionals on the amount of evidence available to support health associations and interventions for gut microbial and gastrointestinal health markers. As research into the gut microbiome and its links with health is rapidly evolving, Microba's SRP will be applied at regular intervals to ensure information in the MetaXplore range of tests and other Microba products remains current.

It is Microba's goal to provide the best tools to precisely measure the gut microbiome and to improve human health through unlocking the complexity of the gut microbiome. It is our hope that Microba's SRP will help healthcare professionals better evaluate and apply the current evidence for microbiome-based associations and interventions to improve management of patient health.

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Appendix A - Expertise and qualifications of Microba scientific reviewers

Name	Expertise	Qualification	Review period
Alena Pribyl	Gut microbiome, molecular biology	PhD	2021-2022 2024
Brad Leech	Nutrition, naturopathy	PhD	2021-2022 2024
Paula Smith Brown	Dietetics (APD), gut microbiome	PhD	2021-2022 2024
Alyssa Tait	Nutrition, physiotherapy, naturopathy	MS	2021-2022
Angela Genoni	Nutrition, food science, gut microbiome	PhD	2021-2022
Belinda Gray	Nutrition, naturopathy	PhD	2021-2022
Laima Hareer	Nutrition, dietetics, naturopathy	BNutrDiet	2021-2022
Annika Krueger	Cell biology, nutrition	PhD	2024
Hannah Naismith	Food science, nutrition	BSc	2024

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